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by

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16. ABSTRACT

A wide variety of electrophysiological techniques are available for use in detection and characterization of neurotoxicity. One of these techniques, the flash evoked potential, or FEP, has been discussed in some detail to provide a forum for raising issues common to many electrophysiological techniques. The issues raised include interpretation of results and methodological variables important for successful use of the techniques. In addition, several little-used but potentially valuable methods were identified.

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Introduction

Comprehensive evaluations of potential neurotoxicants often contain neurophysiological studies. Depending upon the methods selected, neurophysiological measures may be used to achieve goals as diverse as detecting neurotoxicity, characterizing neurotoxicity (e.g. which neural systems are involved) and unraveling mechanisms of neurotoxicity. A review of the relevant literature produces the disappointing finding that relatively few of many potentially useful neurophysiological methods have ever been employed in a toxicological context. The purpose of this chapter is threefold: (1) to review the potential application of Neurophysiological methods that are rarely used in toxicology; (2) to describe Neurophysiological methods that have been used most often in toxicology, providing examples of data obtained using these methods; and (3) to identify relevant methodological and interpretational issues.

Conceivably, any of several hundred available neurophysfological methods could be applied to problems of neurotoxicology. To avoid beginning a task that could not be finished, some limits were placed upon the scope of the present chapter. Given the nature of this book, a decision was made to concentrate upon measures that are relevant to vertebrate behavior. Specifically excluded from discussion are studies of isolated systems (cell culture, slice, etc.), invertebrate studies, and studies generally considered to require anesthesia for their successful completion (e.g. intracellular studies). There is no intent to place a value judgment upon excluded methods. Rather, they are better suited for investigation of the mechanisms of toxicity at a more molecular level than what is

implied by the title of this book. A substantial literature of macroelectrode methods remains from which to choose. Some methods such as kindling (Joy, 1984) and recovery processes (Dyer and Boyes, 1983) have been reviewed recently and therefore will not be discussed here.

In an effort to provide organization and understanding to such a complex integrated organ, it is convenient to divide the brain into discrete functional systems. Thus, one may refer to the visual system, the somatosensory system, the hippocampal system, the extrapyramidal system, etc. While few would argue that these systems are not interrelated, they are almost always treated separately. Most such systems are constructed of neurons with similar membrane and axonal properties, but differ with respect to connectivity, and may differ with respect to transmitters, and metabolic activity. Therefore it is logical to assume that evaluation of any system provides information concerning general CNS effects as well as effects unique to the system under investigation.

Certain systems appear more sensitive to neurotoxicants than others. Often, it is not known whether apparent sensitivity or insensitivity of a system reflects (1) sensitivity of the test method; (2) accessibility of the system to the investigator; (3) accessibility of the system to the toxicant; or (4) relative vulnerability to damage of the system or its constitutents . While considerable work must be done to address this issue for each case, the real message is that the careful investigator will give these issues thought before a particular test method is either adopted or rejected.

In this chapter certain specific systems and methods have been selected for more detailed consideration as general examples of classes of systems (e.g. the visual system as a general example of sensory systems) and methods (e.g. flash-evoked potentials as a general method for assessing the visual system). For any system or method the following questions should be kept in mind. What can we learn from this general approach (i.e. what are the clinical and neurobiological correlates of alterations in the endpoints)? How does one do these experiments most effectively? What have we learned about neurotoxicants from studies which have used this approach? What control factors seem to be particularly important? How feasible is the method, in terms of equipment, personnel and time? What are the limitations of the approach? Answers to some of these questions will be found in the text, others must await further study.

Evoked potentials are among the oldest tools available to the neurophysiologist. Simply defined, they are alterations in voltage recorded from a particular brain area which are time-locked to an evoking stimulus. The recordings are presumed to reflect graded post-synaptic potentials integrated over a large population of neurons. Because they are often small relative to ongoing ("spontaneous") electrical activity, they are customarily averaged over a number of trials. The averaging process improves the signal to noise ratio by a factor of the square root of the number of trials averaged. Much has been written about evoked potentials, and the reader is referred to some extensive treatments for many of the issues which will not be addressed here (Regan, 1972; Dyer, 1984; Rebert, 1983).

Four different classes of evoked potentials will be considered here: those which evaluate sensory systems, those which evaluate hippocampal systems, those which evaluate interhemispheric function, and those which evaluate electrical correlates of cognition.

Sensory evoked potentials are the most commonly recorded. They may be recorded in classically defined sensory systems following either sensory stimulation or electrical stimulation in part of the system. Sensory evoked potentials may also be recorded from brain areas which are not often considered part of sensory systems (e.g. basal ganglia, cerebellum, hippocampus, etc.). The only sensory system evoked potentials to be considered here are those which are elicited by sensory stimulation. Further, the most extensive discussion will be reserved for flash-evoked potentials, since these have been used most extensively in neurophysiological assessments of neurotoxicity.

II. Flash-Evoked Potentials (FEPs) -

Flash evoked potentials are most often elicited by intense but brief (on the order of 10 usec) flashes of light, usually from a strobe. When recorded from the visual cortex of rats, FEPs take on a characteristic appearance (see Figure 1). The primary evoked potential may be defined as consisting of 3 positive and 3 negative peaks, occurring within about 200 msec of the flash. Under certain conditions, the third negative peak is followed by a series of waves, which are known as the photic afterdischarge (Bigler, 1977). FEPs may also be recorded from non-cortical structures, including optic tract, lateral geniculate nucleus and superior colliculus, although such recordings have been less frequently employed in neurotoxicology than those obtained from the visual cortex.

The data obtained from FEP experiments are typically peak latencies, in msec, and either peak-to-peak amplitudes or baseline to peak amplitudes, in μvolts. With 3 positive and 3 negative peaks, there are 6 latencies and 5 peak-to-peak or 6 baseline-to-peak amplitudes which may be obtained, or a total of 11-12 variables. Any or all of these variables may change as a function of exposure to a toxicant, and the task is to interpret the meaning of different patterns of change. While few definitive answers are available from FEP studies of the visual cortex, some educated inferences are possible.

A. Generators of FEP Peaks

Before considering different patterns of change which have been observed, it is important to summarize the current status of our know-

ledge about the generators of the different peaks of the FEP. Defining generators may be done at many different levels. A first and important question concerns whether in fact all of the peaks recorded from electrodes positioned over the visual cortex reflect activity in the visual cortex, or whether the potentials are volume conducted potentials from either subcortical structures or distant cortical structures. This question is best answered by recording FEPs from (a) different cortical loci; and (b) different depths (i.e. a laminar analysis) within the cortex. If the recorded potentials are all of local origin, than as the active electrode is lowered through the cortex the waveform will either reverse polarity or disappear, depending upon location and orientation of the generators within the cortex. Furthermore, locally generated potentials will decrease in amplitude as a function of the square of distance from the source along the cortical surface, and in proportion to the cosine of the angle from the source to the recording point (see Lopes da Silva and Van Rotterdam, 1982), if the source is below the surface.

Formal experimental treatment of the reversal of FEPs recorded from unanesthetized rat visual cortex does not appear in the literature, although several investigators have noted reversal within 1.5 mm from the surface (Ebe, et al., 1972; Hetzler, personal communication; Schwartzbaum et al., 1972). It is also known that in cats the FEP reverses within the cortex (Creutzfeldt and Kuhnt, 1973), and in most reports the assumption that the recorded potentials are of cortical origin is implicit. Furthermore, there is evidence that in the rat, FEPs diminish rapidly with distance from the primary visual areas (Montero, 1973). It thus seems likely that FEPs recorded from electrodes located over rat visual cortex originate in rat visual cortex.

Knowing that FEPs recorded from visual cortex originate in visual cortex is important, but interpretation of alterations in the various components requires further information. The positive and negative waves recorded from the surface could each reflect either excitatory or inhibitory activity, depending upon both the location of the activated cells and the activated portion of them within the cortical layer (Lopes da Silva and Van Rotterdam, 1982). In other words, similar waveforms could be recorded following widely different patterns of activity. Creutzfeldt and Kuhnt (1973) have proposed a cellular model to account for FEPs recorded in the cortex of anesthetized cats, and provide some correlative data to support the model. However an adequate test of the model is difficult, since it would require the simultaneous intracellular recording from a large number of neurons. Furthermore, identification of the mechanism generating a particular component is of limited value until the connectivity of the responsive cell population is established.

It is possible that the cortically recorded FEP provides a passive reflection of the pattern of input from the lateral geniculate nucleus (LGN). Indeed, some post-flash histograms of LGN single cell activity correlate well with cortically recorded peaks N1 and N3 in the rat FEPs [Bigler, 1977]. This suggestion is also supported by the work of Verzeano (1973), who suggests that positive and negative FEP waves are correlated with the probability of discharge of different cortical neurons, which in turn could simply reflect patterns of LGN input.

Assuming that cortical waves N1 and N3 of a rat FEP simply reflect the input from LGN, it is still not certain why the pattern LGN inputs shows

the rhythmicity that it does. Mounting evidence suggests that the pattern of LGN activity is a reflection of recurrent interaction between LGN principal relay neurons and inhibitory interneurons, which may be located within LGN or in nearby loci, such as the thalamic reticular formation (e.g. Standage, Fleming and Bigler, 1981).

Nor is it certain where cortical wave N2 comes from. Several studies in cats have addressed the origins of a "secondary cortical response" elicited by flash stimulation [e.g. Brazier, 1957; Torres and Warner, 1962). Torres and Warner (1962) have described 2 types of secondary responses. The first type is augmented by pentobarbital anesthesia and brainstem transections at the midcollicular level. The second type is eliminated by pentobarbital anesthesia and intercollicular transections. Since the P2N2P3 complex in rats is eliminated by pentobarbital anesthesia (Hetzler and Oakley, 1981), it seems reasonable to assume that this complex is analogous to the type 2 secondary response of Torres and Warner (1962). Therefore, brainstem or midbrain involvement are implicated as generators of this potential. A major pathway of visual information to the brainstem reticular formation is through the superior colliculus (Burne et al., 1981), and it is therefore possible that this component is generated via activity in a collicular-reticular-cortical loop. A further suggestion that this component is derived from collicular activity came from the developmental studies of Rose and Lindsley (1968). In these studies, a portion of the 17 day old kitten waveform recorded from the cortex following flashes could be abolished by lesions of superior colliculus and pretectum. While these data imply an influence of the superior colliculus on this peak, recent studies by Geboff (unpublished) Masters Thesis) demonstrate that massive lesions of superior colliculus

do not abolish the P2N2P3 wave complex in rats. Therefore, the origin of this peak remains elusive.

B. Interpreting Altered FEPs

While knowing the origin of peaks should assist in determining the locus of dysfunction when peak morphology is disturbed, assessing the nature of dysfunction requires more information. The physiological significance of an increase or a decrease in the latency or amplitude of a particular peak must be understood in order to determine the nature of dysfunction. The same effect may be obtained by different mechanisms. Therefore, adequate explanation of results in terms of mechanism will always require further experimentation and analysis. While it should also be pointed out that only rarely are such further analyses beyond the capabilities of a resourceful laboratory, each investigator must weigh the importance of such further analyses in the context of their laboratory's effort. In other words, it is often of major interest to simply identify that toxicity exists in a certain system, and of secondary interest to know the cellular mechanism. Many applied labs are not afforded the luxury of pursuing secondary interests.

Figure 2 illustrates several simple schematic waveforms, which will be used for purposes of discussing toxicant induced alterations. It must be remembered that even such simple waveforms represent integrated responses obtained from perhaps millions of postsynaptic potentials. Any process which systematically disrupts the amplitude, duration or latency of a significant portion of the individual postsynaptic potentials will affect the evoked potential. In the context of a FEP study, we can think of the Fig 2 waveform as representing the P1N1P2 complex.

Among the more common findings in FEP studies of neurotoxicity is that peak latencies are increased. A latency increase may or may not be accompanied by an increase or decrease in amplitude. We shall consider each of these cases separately, beginning with the case in which the only observed alteration is an increase in latency of the wave. The most plausible interpretation of such a finding is that the same population of inputs to visual cortex has been activated as in control conditions, but that the onset of activation has been delayed. Other interpretations are possible (e.g. a different population of inputs has been activated, or the durations and magnitudes of some of the inputs have been changed, but the pattern of change is such that it exactly duplicates the control condition in all but latency), but unlikely because of the extraordinary coincidence they require. If the onset and return to baseline of the wave are shifted by the same amount, then a delay in conduction velocity is a most likely explanation. A disruption in myelin, such as produced by triethyltin produces such a change [Dyer and Howell, 1982].

Another way of evaluating patterns of change in latency of a waveform is illustrated in Figure 3. In this figure the treatment-induced latency change is plotted on the ordinate, and the normal FEP peak latency is plotted on the abscissa. The peaks are separated on the abscissa by a distance which corresponds to their normal separation in time from the stimulus. It is evident that the function describing the points for any given treatment can indicate the magnitude of effect, since this is simply a function of distance on the ordinate. This analysis can also describe the extent to which an effect is evenly distributed within the wave. If the function is linear, and the slope is 0, then the entire wave has shifted by a constant amount. Constant waveform shifts are

unusual in the literature, but have been reported for rats administered daily dosages of 1 mg/kg triethyltin (TET) (Dyer and Howell, 1982). The implication of this finding is that the entire effect of treatment occurred at a distant location, and that the effect was probably upon conduction velocity. In the case of TET, it seems most likely that conduction velocity in the optic nerve was affected (Scheinberg et al., 1966).

One of the most common patterns of change seen in FEPs following exposure to toxic insult is a progressive shift in latency. In other words, each successive peak is shifted by a greater amount than the preceding peaks. A straightforward example of this type of shift may be seen in the case of hypothermia. Figure 3 presents data extrapolated from a recent study in which FEPs were recorded from anesthetized rats at different rectal temperatures (Dyer and Boyes, 1983). The figure illustrates several interesting features. First, all functions appear linear. Second, as extent of hypothermia increases from 2 to 6 below normal, the functions shift in both intercept and slope. What this means is that the magnitude of treatment (hypothermia) determines the magnitude of difference between early (P1) and later (P2) peaks. A variety of mechanisms might be invoked to explain this pattern of change. The simplest and most plausible mya be found in the work of Westerfield et al. (1978), who showed that in single nerve fibers, progressive hypothermia produces an increase in the duration of an action potential, a decrease in its conduction velocity, and an increase in the length of its relative refractory period. This combination of effects could easily produce the pattern of change produced by hypothermia in the FEP.

Hypothermia accompanied by anesthesia also produces an increase in FEP amplitudes (Dyer and Boyes, 1983). While it is tempting to speculate that an increase in amplitude of a population response like the FEP could reflect release of synaptic inhibition, it is noteworthy that increases in amplitude also occur in single fiber preparations with hypothermia (Westerfield et al., 1978), thereby rendering synaptic explanations unnecessary (although not necessarily incorrect).

The frequency with which the above pattern of change is seen in FEP latencies following exposure to different agents raises the suspicion that a common underlying mechanism is at work. Similar progressive changes in FEP peak latency are observed with ¶-methyl para tyrosine (Dyer et al., 1981), chlordimeform (Boyes and Dyer, 1984), and sulfolane (Dyer et al., 1984).

There are, however, distinctly different patterns in latency change produced by some compounds. One such pattern is evidenced by rats treated with trimethyltin (TMT), an organotin known to produce loss of some retinal ganglion cells (Chang and Dyer, 1984). TMT-treated rats have FEPs with slightly longer than normal P1 and N1 latencies and slightly shorter than normal P2 latencies. Such a pattern, coupled with a reduced amplitude of the potential, as occurs in TMT-treated rats (Dyer et al 1982), is congruent with a reduction in the population of cells responding to the stimulus. However without further studies it would not be possible to determine where the reduction occurred. In the case of TMT, further studies indicated that retinal cells were being lost as a result of exposure (Dyer et al., 1982). Decreased latency of N1 and P2 with no change in P1 and no change in amplitude has also been observed

in rats exposed pre-orperinatally to lead (Burdette, 1983). The latter finding was surprising in light of the report by Fox et al (xxxx) that perinatal exposure to lead produces enormous increases in P1, N1 and P2 latencies. Decreased latency of P2 coupled with increased P1N1 and N1P2 amplitudes has also been observed with prenatal exposure to methyl mercury (Dyer, Eccles and Annau, 1978).

There are very few treatments which have been reported to produce amplitude alterations in the absence of latency alterations. However prenatal administration of carbon monoxide to female rats (Dyer et al., 1979) produces increases in P1N1 and N1P2 amplitudes without changing latencies. Increasing stimulus (flash) intensity produces a more pronounced increase in amplitudes than decrease in latencies (Dyer et al., 1978).

Changes in amplitude, with or without changes in latency, are difficult to interpret. An increase in amplitude may occur at the single membrane level (as seen in hypothermia, above), as a result of increased synchrony in response to the stimulus (for example if spontaneous activity was diminished, such that fewer neurons were in the refractory period from spontaneous activity when the stimulus was presented), or as a result of removal of inhibition (as seen in evoked potentials recorded from dentate gyrus following perforant path stimulation in kainic acidtreated [Sloviter and Damiano, 1982] and TMT-treated [Dyer et al., 1982] rats). While it also seems likely that increases in amplitude might occur as a result of increased excitability of the neurons, the evidence is conflicting. A study of picrotoxin, strychnine and pentylenetetrazol failed to reveal any increased amplitudes in P1N1 or N1P2 of FEBs

(Bigler et al., 1978), but pentylenetetrazol did increase amplitude of primary auditory evoked responses (Faingold, 1978).

Patterns of change in the P1N1P2 component of the FEP in addition to those discussed above are possible, but those mentioned above have been most commonly observed in FEP studies of neurotoxicity. As the FEP wave discussed becomes further removed from the primary (P1N1P2) component, the interpretation of the different patterns of change becomes progressively more difficult. At present, there is little upon which to base explanations of selective alterations in the P2N2P3 component. Interpretation of changes in this wave would be greatly enhanced by an understanding of the origin of inputs which produce the wave.

More information is available for interpretation of the P3N3 wave of the FEP than the P2N2P3 wave. This wave is occasionally considered to be the first wave in the photic afterdischarge, although the evidence to support this contention is contradictory [e.g. Bigler, 1975; and Bigler et al., 1978]. In any case, alterations in amplitude of the wave appear related to such variables as arousal, habituation and excitability. On a continuum from anesthesia to seizures, both extremes seem to depress P3N3 amplitudes. Thus, reduced amplitudes have been observed following administration of the convulsant strychnine (Bigler et al., 1978), the anesthetic sodium pentobarbital (Hetzler and Oaklay, 1981), the stimulant amphetamine (Fleming et al., 1974, and Schwartzbaum et al., 1974), the anticonvulsants sodium valproate (Myslobodsky and Morag, 1981) and dipropylacetic acid (Shearer et al., 1978), and the cortical activator physostigmine (Fleming et al., 1974; Bigler et al., 1978). Rats with a normally high activity level had smaller P3N3 amplitudes than rats with a noramlly low activity level (Joseph et al., 1981).

On the other hand, P3N3 amplitudes have been increased by procedures which (1) decrease behavioral reactivity to the flash (e.g. Schwartzbaum and Kreinick, 1973); (2) tend to promote drowsiness, such as haloperidol administration (Dyer et al., 1981); (3) might be related to habituation, such as session number within a series of sessions (e.g. Schwartzbaum and Kreinick, 1973; Schwartzbaum and Kreinick, 1974; Schaefer et al., 1974; but not Bartus and Ferris, 1974); and (4) produce EEG hypersynchrony, such as |-vinyl GABA (Myslobodsky and Morag, 1981). Pentylenetetrazol, a convulsant, has been reported to increase P3N3 amplitudes on some occasions (Bigler, 1975) but not others (Bigler et al., 1978).

From the above discussion it should be evident that although interpretation of FEPs is complex, there are certain patterns which emerge and which tend to characterize certain kinds of changes in neurobehavioral function. It should be indicated here that while FEPs are still only rarely used in studies of toxicity, there is at least one major form of neurotoxicity which was first described as a result of a FEP study. Trimethyltin (TMT) is an organotin which was known to produce profound damage to neurons in the limbic system, with somewhat less damage elsewhere (e.g. Brown et al., 1979; Chang et al., 1982; and Dyer et al., 1982). As part of the process of validating the FEP, some TMT-treated rats were tested. Although the only existing study describing TMT-induced neuropathology specifically excluded the retina as having TMT-induced damgage (Brown et al., 1979), the FEPs recorded from treated animals had a decreased amplitude P1N1P2 peak, which was narrower than normal (i.e. increased P1 latencies, decreased P2 latencies). Such a pattern would be expected if fewer fibers were contribut-

ing to the evoked potential. Subsequent work, recording FEPs from the optic tract of TMT-treated rats, showed that these changes occurred at the retinal level (Dyer et al., 1982), and a closer look at the retina of TMT-treated rats revealed that indeed there was a loss of retinal ganglion cells (Chang and Dyer, 1983). As a result of this study, a series of other studies were performed which indicated that in fact TMT produced widespread damage to other sensory systems as well (Chang and Dyer, 1983).

C. Methodological Considerations

The above discussion has addressed some of the interpretation issues involved in FEP studies. There are also many methodological issues of concern. In most cases the optimal conditions under which to perform a FEP study are not known, and the purpose here is to simply identify the issues. It would be surprising if there were not particular sets of conditions under which performance of FEP studies would be more likely to detect toxicity than others. While on the surface it might appear desireable to systematically manipulate the conditions of recording in routine testing, thereby obtaining for any given toxicant the set of conditions which is most useful, the magnitude of doing such experiments, to say nothing of the complexity of the subsequent multivariate analyses, is enough to intimidate even the most energetic of investigators. Furthermore, optimal conditions may vary with the compound being tested, and may be dictated by a specific hypothesis being tested. In identify these issues, it is hoped that some readers will be tempt-

ed to address the question of what sets of conditions is most likely to detect neurotoxicity.

Stimulus variables - In performing any evoked potential study, a decision about the modality and type of stimulus must be immediately followed by a decision about the appropriate stimulus intensity. In FEP studies, the question becomes how bright a flash should be used. A priori hypotheses may be developed to explain why either a very bright or a very dim flash might be most useful, but there is little data to support either view. Similarly, one can expect to record very different evoked potentials, depending upon whether the rats are in a brightly illuminated environment, a dark environment, or something in between. No FEP studies have addressed the importance of background illumination in detection of neurotoxicity. The interstimulus interval (ISI) is a variable which the investigator has a vested interest in keeping as short as possible. The shorter the ISI, the more rapidly the study can be completed. Unless the point of the study is to examine recovery processes, ISIs for FEPs should be at least 1 sec, because the system takes at least this long to recover from a preceding flash. However beyond 1 sec it is not clear whether increases in ISI render the system more or less sensitive to detection of toxicity.

Animal variables - There are even more options to exercise in selecting the animal and its condition than there are for selecting the stimulus to use. Once the species has been selected, there is still a myriad of other decisions which must be made. For example, if rats are selected, then a strain must also be selected. Even the selection between

albino vs hooded rats is not straightforward. Hooded rats are presumed to have better visual systems, but does this mean they would be more or less susceptible to visual toxicity? A more developed system might have more redundancy built into it, such that small changes would not be noticeable. On the other hand, a vestigial system might be exquisitely sensitive, because if any part of the system is affected by the toxicant, the whole system is rendered nonfunctional. As with stimulus intensity, there are virtually no data to address this question. Most studies have been performed upon Long-Evans hooded rats, with the presumption that results have more generality to other mammals than would results obtained from albinos (Creel, 1980).

Most investigators select either males or females for study. It is rare that a study uses both, since including gender as a factor virtually doubles the size of the study. Yet there are a number of cases in which toxicity is gender-dependent (e.g. Dyer et al., 1979). More often than not, males are used, presumably because it is thought that the estrous cycle in females would increase variability and thereby reduce sensitivity. While there are known gender differences in FEPs (Dyer and Swartzwelder, 1978), persuasive evidence that males produce less variable FEPs than females is lacking.

Another variable to consider in the selection of animals for a FEP study is size. Leaving alone the differences between developing rats and adult rats, there are still decisions to be made with regard to size of adult rats to use. In particular, a common (although unpublished) observation from our laboratory has been that even amond adult rats, there

is a correlation between weight of the animal and certain parameters. In a recent sample of 52 control male hooded rats from several different experiments, ranging in weight from 313 to 545 gm, there was a correlation between body weight and FEP N1 latency of -0.452 ($p<0.0008$). In this particular set of animals, there was also a small correlation between weight and P3 latency (-0.33, $p< 0.018$), but no significant correlation between weight and any amplitudes. While it is not clear whether the critical variable is weight or age, since these are confounded in male hooded rats, it seems obvious that one should strive to maintain uniform weights within a given study. Alternatively, if weights are obtained prior to an acute treatment, one might use pretreatment weight as a covariate in analysis of the data.

Following selection of the desired species, strain, gender and size of animal to study, there are several considerations in treatment of the animals, independent of the toxicant under study, which may be expected to influence the characteristics of FEPs. Perhaps most obvious is location of the recording electrode and the reference electrode. While it is clear that location per se influences response amplitude, it may also be the case that there are location-dependent toxicities, even within the visual cortex. This does not seem likely, but it has never been tested. In addition to the placement, the amplitude of recorded responses may be affected by the extent to which electrodes penetrate the skull, dura and cortex. While there are no data from which to argue that any particular configuration is optimal, uniformity and standardization should again be the rules for any given laboratory.

At the time of recording, many investigators consider it imperative

to dilate the pupils with atropine, to reduce the animal's capacity to vary effective stimulation by pupillary constriction. In actual practice, the presence or absence of topical atropine makes little difference unless the compound under study produces effects upon pupil dilation. If the rats are not expected to perform a visual task, but are rather only receiving flash stimuli, then nothing is lost by routinely dilating the pupils.

A variable which has only recently begun to appear in the literature as relevant in FEP studies is ambient temperature. It is well known that alterations in body temperature affect FEPs (e.g. Dyer and Boyes, 1983), but it is less well appreciated that many toxicants alter body temperature in a manner which is contingent upon the ambient temperature (e.g. Dyer, and Howell, 1982; Myers, 1981). We have recently observed that ambient temperature fluctuations over just a few degrees can alter rectal temperature of control rats. This was most dramatically revealed when control rats placed on the top shelf of a three shelved laboratory cart had rectal temperatures which were about 1C above those placed on the bottom shelf (unpublished observation).

Two final variables we shall consider which pertain to the state of the animal at the time of recording are habituation and arousal. Some investigators routinely employ a fixed habituation time following placing an animal in the experimental setup prior to initiating recording. While there are few published accounts describing the influence of habituation upon FEP parameters (see e.g. Bartus and Ferris, 1974), it is certain that habituation time affects some of the peaks, especially the amplitude of late components. It seems likely that one might find an optimal

habituation time which balances the need to maximize peaks amplitude with the need to record data as rapidly as possible.

A rat which has not been handled and which is placed in the experimental recording chamber for the first time may be assumed to be in an aroused condition, at least for a brief period of time. However as the test session increases in duration, or as the animal gains repeated experience with being handled and/or being placed in the test chamber, one may expect an increased probability that sleep will occur. It is known that evoked potentials differ in sleeping and waking subjects (e.g. Demetrescu et al., 1965; Guilbaud et al., 1972), and therefore rats with fluctuating wakefulness add considerable variability to the data. One solution to the problem has been adopted by Winneke (1979), who routinely record only when rats are asleep. It may be that FEPs obtained during one particular stage of sleep or wakefulness may be more sensitive to toxicant-induced disruption than others, but no systematic studies have yet been published which address the problem.

Analysis Variables - The chapter by Muller in this volume addresses the major design issues which face investigators wishing to perform FEP (or any) studies. While an investigator may consult a statistician for assistance with designing the study, the investigator must take responsibility for selection of the endpoints. There are several unresolved issues regarding the most useful endpoints in FEP studies. The most common strategy is to record peak latencies and peak to peak amplitudes, producing a total of 11 variables (P1, N1, P2, N2, P3 latencies, P1N1, N1P2, N2P3 and P3N3 amplitudes). However some investigators prefer measuring baseline to peak amplitudes (e.g. Hetzler and Oaklay, 1981).

Peak to peak amplitudes provide l less variable than baseline to peak, and may be less variable than baseline to peak (Dyer and Annau, 1977). On the other hand, baseline to peak measures may detect more subtle alterations in waveform induced by a toxicant, if the neural determinants of the two peaks involved are distinct. Some direct comparisons of the two approaches would be useful. Perhaps because of interpretation problems, the neurotoxicology literature has not seem much in the way of fourier analysis of waveforms or integrated amplitudes raather than individual peak amplitudes. Rebert (personal communication) has suggested that the RMS amplitude of the entire waveform provides an effective summary of brainstem auditory evoked response (BAER) amplitudes. While these methods might take the investigator further from the raw data, they may provide fewer endpoints as well. Potentially a combination of the two sets of approaches could be useful.

III. Other Sensory Evoked Potentials

While FEPs have been the sensory evoked potentials most studied in the context of neurotoxicology, there are certainly many applications where other methods might be more useful. Even for testing visual function, it is easy to argue for more sophisticated methods. One method which has been used extensively in the neurological clinic and is beginning to be used in neurotoxicology is the pattern reversal evoked potential (PREP). As has been described recently (Dyer, 1984), the PREP, the brainstem auditory evoked response (BAER) and the somatosensory evoked potential (SEP) all have unique contributions to make to an electrophysiological evaluation of neurotoxicity. While none of these tests has the extensive background of toxicological and methodological studies which

the FEP enjoys, the recent literature suggests that this will not long be the state of affairs (e.g. Rebert, 1980; Rebert et al., 1982; Rebert et al., 1983; Janssen et al., 1983; Boyes and Cooper, 1981; Arezzo et al., 1982; Boyes and Dyer, 1983; Boyes and Dyer, 1984; Boyes et al., 1984).

When the goal of a study is to assess the functional integrity of a particular sensory system, then it makes most sense to record from either relay or cortical areas presumed to be primary parts of the system. For example, the optic nerve, lateral geniculate, visual cortex and superior colliculus are logical places from which to record in the visual system. However if the experiment is less concerned with determining the functional integrity of a particular system, and more concerned with the functional integrity of the whole nervous system, then an alternative strategy is to record the response to sensory stimuli from structures which are not traditionally considered part of the primary sensory systems. The sensory stimulus is the route into the brain, and in some respects the more polysynaptic the route to the recording electrode, the more likely that alteration of some brain function will be detected by the technique. In this context, one might also record from sensory structures like the visual cortex while delivering stimuli within another modality, such as the auditory modality.

While this approach is logical, there have been few attempts to follow it. Perhaps one reason for the reluctance of investigators to utilize this approach is that recordings from subcortical structures bring with them the onus of demonstrating that the recordings were in fact from the desired area. While histological verification of electrode placement is routine in these studies, this in not always sufficient. For example, an often recorded site is listed as the midbrain reticular

formation. However given the massive input of sensory information to the overlying superior colliculus, an investigator must demonstrate that his responses are not volume conducted from above. The same may be said for responses evoked by visual stimuli and recorded in hippocampal formation overlying the lateral geniculate nucleus or superior colliculus. To date there have been no convincing demonstrations in the toxicology/ pharmacology literature that effects reported were not volume conducted.

Another possible reason for the relative absence of subcortical recordings in toxicological literature is that using them virtually demands a within subjects design for the study. Variations in morphology and amplitude occur with even the slightest variation in depth of electrode placement (obviously recordings made from the brain surface do not suffer from this problem). Thus, with the added source of variability imposed by depth placements, a considerably larger number of subjects must be included to render between group differences statistically significant.

Most attempts to use the subcortical and/or nonsensory approach have been in the pharmacological literature (although see Ray, 1981). One laboratory which has pursued this approach in a pharmacological context is that of Dafny and coworkers (e.g Dafny and Rigor, 1978; Dafny, 1978; Schanzer et al., 1978; Rabe et al., 1980). In these studies recordings have been made from electrodes in structures like the ventromedial hypothalamus, the centromedian, caudate, medial forebrain bundle, midbrain reticular formation, but no convincing demonstration that potentials were not volume-conducted from primary structures has been presented. Compounds tested have included ketamine, sodium pentobarbital, pro-

staglandin E2, and halothane. At present no definitive statement may be made about the relative sensitivity of these recordings compared to the more traditional sensory recordings from sensory structures.

IV. Hippocampal Evoked Potentials

In the last 20 years, it seems that no area of the mammalian brain has attracted more attention than the hippocampus and its connections. There are certainly many reasons for this attention, including its laminar anatomy, its defined input-output pathways and its apparent involvement in such complex neurobiological processes as memory and epilepsy. The hippocampus is clearly an extremely important brain area, and for no other reason is deserving of considerable attention from neurotoxicologists. In addition to its general importance in brain function (or perhaps related to it), the hippocampus also appears to demonstrate an intriguing tendency to (1) accumulate some toxic substances; and (2) be damaged more than some other structures by certain toxic insults. For these reasons, it is important to consider the potential utility of hippocampal evoked potentials as measures for detection and characterization of neurotoxicity.

Before considering the few occasions upon which hippocampal EPs have been used in a toxicological assessment, it is important to have a better appreciation for what makes the hippocampus unique. A detailed treatment of these factors is beyond the scope of this chapter, but they will be identified here.

Much of what makes the hippocampal formation unique may be treated to its anatomy. While the cross sectional anatomy of the hippocampus

appears simple, it is the third dimension which provides complexity. Many adequate descriptions of hippocampal anatomy exist [e.g. Lorente deNo, 1934; O'Keefe and Nadel, 1978; Swanson, 1979]. In cross section the hippocampal formation may be thought of as two thin sheets of tightly packed cells, one in the shape of a partially flattened "C" and the other in the shape of a backwards partially flattened "C". The two "C"s face each other, the bottom limb of one entering the opening of the other. The entered "C" is the sheet of dentate granule (DG) cells, the entering "C" is the sheet of pyramidal cells, forming what is called ammon's horn, or the cornu ammonis (CA). The CA is divided into cell fields, depending upon location along the sheet. Those cells at the top of the CA "C" are the CA1 cells. Most of the other CA cells are CA3 cells, except for a few towards the top of the curvature which are CA2, and a few loose ones inside the DG "C", which are CA4. The major connections of these cell groups are also quite simple. The primary input to the hippocampal formation (aside from contralateral hippocampal and septal) is from the entorhinal cortex via a pathway known as the perforant path. The perforant path provides excitatory input to the DG cells. The DG cells send axons known as mossy fibers which synapse onto CA3 pyramidal cells. The CA3 pyramidal cells send axons out of the hippocampus via the fornix, but also to the CA1 pyramidal cells via the Schaffer collaterals. Both CA1 and CA3 are subdivided into 3 sections, CA1a, CA1b, CA1c, CA3a, CA3b, and CA3c, according to their distance along the sheet of cells from the border between CA1 and the subiculum. These different sections of the subfields have pyramidal cells with somewhat different properties, some tending to fire in burst discharges, others tending to fire in single spikes [Masukawa et al., 1982]. The DG, CA3 and CA1 cells all have nearby

populations of basket cells, which provide recurrent inhibition.

As mentioned above, the anatomy of the hippocampus is complex in the third dimension. In fact, the "C"s described above are really more like two troughs or gutters, extending from the septal area (septal pole) in a posterior-lateral direction at about a 45° Angle from the midline. While the lateral shift continues until the end of the hippocampus, the posterior movement stops after several mm (in the rat), and the two interlocking "C"s swing down (ventral) and forward (anterior). Thus, viewed in 3 dimensions, each of the "C"s has the shape of a ram's horn. This third dimension is known as the septo-temporal axis, and is important because certain toxicants produce different types of damage at different locations along the axis (Chang and Dyer, 1984), and cells at different levels along the septo-temporal axis receive input from different areas and with different densities [Gaarskjaer, 1978; Ruth et al., 1982].

The anatomical organization of the hippocampus renders it particularly accessible to investigation because it is laminar. That is, an electrode may be placed in a particular layer, and the investigator may be assured of knowing the inputs and outputs of that layer. For example, an electrode placed just above the layer of granule cells in the dentate gyrus would record almost exclusively inputs to dentate granule cells. If an input pathway was stimulated, it might be assumed with some certainty that the recorded response arose from dentate dendrites or nearby somas. This anatomy has also opened the way for exploration of recurrent connections. Again using the dentate granule cells for example, it is known that collaterals of their axons synapse example, onto inhibitory basket cells, which in turn inhibit the granule cells via a monosynaptic

route. Using a paired pulse paradigm, it is therefore possible to examine the functional integrity of the recurrent inhibitory pathway. A single shock to the perforant path produces a large EPSP in the granule cells, which contains a spike at intense enough stimuli. However since this spike reflects summated action potentials in the granule cells themselves, it may be inferred that the spike produces activity in the basket cells, which in turn inhibit the granule cells. Therefore, a second shock, occurring shortly after the first, should produce a smaller spike response, because some of the granule cells would be inhibited. This recurrent inhibition, first explored physiologically by Anderson et al. (1966) with the paired pulse paradigm, has been recommended as a model system for assessing drugs which affect synapses mediated by GABA, the putative transmitter at the basket cell- granule cell synapse [Matthews et al., 1981].

A feature of hippocampal physiology which may be either an asset or an impediment to EP studies, depending upon the design and approach to the study, is the extraordinary plasticity of synaptic relationships. There are at least three types of short term plasticity (paired pulse potentiation, frequency potentiation, and heterosynaptic potentiation (cooperativity)) and three types of long term plasticity (post tetanic potentiation, long term potentiation and kindling) in the hippocampus. While the integrity of any of these models of plasticity may be studied as a function of exposure to toxicants, if the investigator is not interested in plasticity but rather in the "normal" synaptic relationships between areas, then extra care must be taken to avoid placing the hippocampus into the "plastic" mode and inadvertently altering its physiology. The simplest way to avoid error is to utilize a low stimulation rate

(e.g. 1 pulse/sec or less).

A significant impediment to the use of hippocampal evoked potentials in toxicology is the requirement that electrode placements be absolutely precise if amplitude measurements are to be made and compared across subjects. This requirement is a result of the laminar organization of the structure, which produces large fluctuations in amplitude with small changes in depth. However this is not a problem if either (1) the design of the study can be a within subjects design; or (2) some form of normalization of the endpoint can occur. An example of the latter is the case in which an investigator is interested in studying recurrent inhibition. In this case, the endpoint is usually the ratio of the second of a pair of responses to the first, a situation which automatically cancels out absolute differences in amplitude of the first response between subjects. Successful use of in vivo hippocampal evoked potentials in a toxicological context have tended to use a derived measure which normalizes the endpoint across animals.

For example, Abraham et al. (1981) have shown that urethane-anesthetized rats tested 8 weeks after cessation of a 20 week chronic ethanol treatment had normal input-output relationships for EPSPs and population spikes recorded from CA1 after stimulation of the Schaffer collateral system. However treated rats had greater paired pulse potentiation responses for the population spike than did controls (response measure was response 2 amplitude/response 1 amplitude, or R2/R1). These results were interpretted as a reduction of recurrent inhibition. Jordan and Clark (1983) showed that adult rats which were undernourished during the prenatal and early postnatal period had reduced magnitude and duration

of long term potentiation produced in the dentate gyrus by stimulation of the perforant path. The response measure was percentage increase in population spike amplitude compared to pre potentiation baseline. Dyer and Boyes (1984) have demonstrated that within hours of a single acute administration of trimethyltin, there is a reduction of recurrent inhibition in the dentate gyrus, measured by paired pulse stimulation of the perforant path. In this study the response measure was spike amplitude, R2/R1. In a related investigation of trimethyltin, Hasan et al (1984) demonstrated altered responses in the dentate gyrus following polysynaptic activation.

While not strictly toxicological, several studies have used in vivo hippocampal evoked potentials as endpoints in studies of aging and senescence. Landfield et al. (1978) recorded from CA1 in aged urethane-anesthetized rats following stimulation of the commissural-Schaffer collateral system. Aged rats did not differ from controls on such measures as stimulus threshold, response latency, response amplitude or time course of paired pulse effects. However compared to controls, aged rats did not show good frequency potentiation at 12 Hz, and further they demonstrated a slowed rise of post tetanic potentiation after a 5 sec 100 Hz train of stimuli. Barnes (1979) recorded from the dentate gyrus of senescent rats following stimulation of the perforant path. In this unanesthetized preparation, with recordings taken from different loci than those of Landfield et al. (1978), the outcome was somewhat different. Older rats had smaller EPSPs than controls. In addition, older rats showed an impaired response to single stimuli after a series of 20 msec bursts of 400 Hz stimuli. Unlike controls, aged rats did not develop an enhanced facilitation with repetition over days. The first day that high frequency

stimuli were presented, the responses of both control and aged rats were potentiated by about the same amount, and for about the same time period. By the third day of such treatment, however, the potentiation observed in controls was greater and of longer duration than in aged rats. Paired pulse stimulation did not differentiate between aged and control rats at interpulse intervals between 7 and 95 msec.

V. Interhemispheric Evoked Potentials

Another technique which has been underutilized in toxicological investigation is the interhemispheric evoked response, or transcallosal response (TCR). If an area of the cerebral cortex is electrically stimulated, the homotopic area on the contralateral side responds with an evoked response (Curtis, 1940). The response is presumably mediated via the corpus callosum, although under certain circumstances a late response mediated via a subcortical pathway, the interhemispheric delayed response, may also be observed (Rutledge and Kennedy, 1960; Rutledge, 1965).

The TCR gives the investigator a measure of the functional integrity of a cortical tract in a monosynaptic system (Pickworth et al., 1977). While this tract has been used for a variety of purposes (e.g. Preston et al., 1983; Swadlow, 1982), it has yet to be explored adequately in a toxicological context. In rats the development of the TCR has been studied (Seggie and Berry, 1972), and it has been demonstrated that its development is impaired by thyroidectomy (Hatotani and Timiras, 1967) and protein malnutrition (Forbes et al., 1975). Howell has shown that the TCR is impaired by prenatal hexane treatment and prenatal cadmium treatment (Howell, 1978).

VI. Cognition-related potentials

If one includes human studies, there is an enormous body of literature dedicated to the search for evoked potentials, or event-related potentials which are in some way related to cognitive processes. Included among these potentials is the contingent negative variation, a slow potential which develops following an initial stimulus when the initial stimulus is supposed to signal the initiation of a time interval prior to an "imperative" stimulus, one which requires an act, motor or cognitive. These potentials have been recorded in humans and non human primates (Rebert,1972) , and similar waves have also been reported in rodents (Pirch, et al., 1981). The significance of these waves has been debated for nearly 20 years, but there seems to be some general agreement that they are in some way related to "expectancy", that is, the subject is expecting the second stimulus.

In contrast to the expectancy wave, others have studied a much more rapid process which seems to be related to surprise or novelty. This wave, generally referred to as the P300 wave since it occurs at a latency as great or greater than 300 msec, is largest when a stimulus appears which is different from a preceding string of stimuli, and which has been given some meaning by the experimenter. For example a subject might be asked to correctly count the number of "rare" stimuli in a series of frequent stimuli in order to correctly complete a task. Evoked potentials recorded to the rare stimuli would have the large positive peak at around 300 msec, whereas evoked potentials recorded to the frequent stimuli would have at best a small peak. Attempts to produce P300 waves in primates have been successful (Arthur and Starr, 1984), but there is only

a fragile literature on these waves in rodents (O'Brien, 1982). Deadwyler et al. (1981) have recorded waves with similar properties which appear to originate in the dentate gyrus of the hippocampus, and a few human studies also indicate that the hippocampal formation is a viable candidate for generator of P300 (Halgren et al., 1980; Okada et al., 1983). If this can be demonstrated conclusively, perhaps other tests of hippocampal function, mentioned above, might be more readily implemented in animals and provide similar information.

The CNV and the P300 paradigms, as well as several variants (e.g. Otto et al., 1981) provide potentially useful culture-free indicies of cognitive function in humans, which might be particularly useful in evaluations of neurotoxicity. If these turn out not to be redundant with measures of hippocampal function in rodents, then it would be appropriate for investigators to turn attention to developing rodent models for them as indicies of neurotoxicity.

Summary

In summary, a wide variety of electrophysiological techniques are available for use in detection and characterization of neurotoxicity. One of these techniques, the flash evoked potential, or FEP, has been discussed in some detail to provide a forum for raising issues common to many electrophysiological techniques. The issues raised include interpretation of results and methodological variables important for successful use of the techniques. In addition, several little-used but potentially valuable methods were identified. While the relationship between the endpoints discussed and behavioral alterations has been left to the reader's imagination, it is evident that certain of the endpoints are very closely tied

to behavior. It is hoped that this chapter will stimulate interest in pursuing the behavior-neurophysiology interface within the context of toxicology.

VII. REFERENCES

Abraham, W.C., B.E. Hunter, S.F. Zornetzer and D.W. Walker (1981) Augmentation of short term plasticity in CA1 of rat hippocampus after chronic ethanol treatment. Brain Res., 221: 271-287.

Arezzo, J.C., H.H. Schaumburg, H.G. Vaughan, Jr., P.S. Spencer and J. Barna (1982) Hind limb somatosensory evoked potentials in monkey: The effects of distal axonopathy. Ann. Neurol., 12: 24-32.

Arthur, D.L. and A. Starr (1984) Task-relevant late positive component of the auditory event-related potential in monkeys resembles P300 in humans. Science 223: 186-188.

Barnes, C.A. (1979) Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. J. comp. physiol. Psychol. 93: 74-104.

Bartus, R.T. and S.H. Ferris (1974) Neural correlates of habituation and dark adaptation in the visual cortex of the rat. Physiol. Psychol. 2: 55-59.

Bigler, E.D. (1975) Lateral geniculate multiple-unit activity related to metrazol potentiated after-discharges. Electroencephalog. clin. Neurophysiol., 39: 491-497.

Bigler, E.D. (1977) Neurophysiology, neuropharmacology and behavioral relationships of visual system evoked after-discharges: a review. Biobehav. Rev. 1: 95-112.

Bigler, E.D., D.E. Shearer, R.E. Dustman and D.E. Fleming (1978) Differential effects of convulsants on visually evoked responses in the albino rat. Pharmacol. Biochem. Behav., 8: 727-733.

Boyes, W.K. and G.P. Cooper (1981) Acrylamide neurotoxicity: effects on far-field somatosensory evoked potentials in rats. Neurobehav. Toxicol. Teratol., 3: 467-490.

Boyes, W.K and R.S. Dyer (1983) Pattern reversal and flash evoked potentials following acute triethyltin exposure. Neurobehav. Toxicol. Teratol. 5: 571-577.

Boyes, W.K. and R.S. Dyer (1984) Chlordimeform produces profound, selective and transient changes in visual evoked potentials of hooded rats. Exper. Neurol. X:XXX-XXX.

Boyes, W.K., D.E. Jenkins and R.S. Dyer (1984) Chlordimeform produces contrast-dependent changes in visual evoked potentials of hooded rats. (in preparation).

Brown, A.W., W.N. Aldridge, B.W. Street and R.D. Verschoyle. (1979) The behavioral and neuropathologic sequelae of intoxication by trimethyltin compounds. Am. J. Pathol., 97:59-82.

Burdette, L.J. (1983) The effects of asymptomatic lead exposure during different developmental stages in rats: behavior and electrophysiology. (unpublished doctoral dissertation)

Burne, R. A., S. A. Azizi, G. A. Mihailoff and D. J. Woodard (1981) The tecto pontine projection in the rat with comments on visual pathways to the basilar pons. J. Comp. Neurol., 202:287-307.

Chalupa, L.M., Anchel., M.N. and D.B. Lindsley. (1973) Effects of cryogenic blocking of pulvinar upon visually evoked responses in the cortex of the cat. Exp. Neurol., 32: 112-122.

Chang, L.W., T.M. Tiemeyer, G.R. Wenger and D.E. McMillan (1982) Neuropathology of mouse hippocampus in acute trimethyltin intoxication. Neurobehav. Toxicol. Teratol. 4: 149-156.

Chang, L.W. and R.S. Dyer (1983) Trimethyltin induced pathology in sensory neurons. Neurobehav. Toxicol. Teratol. 5: 673-696.

Creel, D. (1980) Inappropriate use of albino animals as models in research. Pharmacol. Biochem. Behav. 12: 969-977.

Creutzfeldt, O.D. and Kuhnt, U. (1973) Electrophysiology and topographical distribution of visual evoked potentials in animals. In, R. Jung (ed.) Handbook of Physiology, Vol II/3, Springer-Verlag.

Curtis, H.J. (1940) An analysis of cortical potentials mediated by the corpus callosum. J. Neurophysiol., 3: 414-422.

Dafny, N. (1978) Neurophysiological approach as a tool to study the effects of drugs on the central nervous system: dose effect of pentobarbital. Exp. Neurol., 59: 263-274.

Dafny, N. and B.M. Rigor (1978) Dose effects of ketamine on photic and acoustic field potentials. Neuropharmacol., 17: 851-862.

Deadwyler, S. A., M. O. West, and J. H. Robinson (1981) Evoked potentials for the dentate gyrus during auditory stimulus generalizations in the rat. Exp. Neurol. 71:615-624.

Demetrescu, M., M. Demetrescu and G. Iosif (1965) The tonic control of cortical responsiveness by inhibitory and facilitatory diffuse influences. Electroencephalog. clin. Neurophysiol. 18: 1-24.

Dyer, R.S. (1985) The use of sensory evoked potentials in toxicology. Fundamental and applied Toxicol. X:xxx-xxx.

Dyer, R.S. and Z. Annau (1977) Flash evoked potentials from rat superior colliculus. Pharmacol. Biochem. Behav. 6: 453-459.

Dyer, R.S. and W.K. Boyes (1984) Trimethyltin reduces recurrent inhibition in rats. Neurobehav. Toxicol. Teratol. (in press).

Dyer, R.S. and W.K. Boyes (1983) Hypothermia and chloropent anesthesia differentially affect the flash evoked potentials of hooded rats. Brain Res. Bull. 10: 825-831.

Dyer, R. S. and W. K. Boyes (1983) Use of neurophysiological challenges for the detection of toxicity. Fed. Proc. 42:3201-3206.

Dyer, R.S. and W.K. Boyes (1984) Sulfolane impairs visual function. Toxicologist, 4: 184.

Dyer, R.S., C.U. Eccles and Z. Annau (1978) Evoked potential alterations following prenatal methyl mercury exposure. Pharmac. Biochem. Behav., 8: 137-141.

Dyer, R.S., C.U. Eccles, H.S. Swartzwelder, L.D. Fechter and Z. Annau (1979) Prenatal carbon monoxide and adult evoked potentials in rats. J. Environ. Sci. Health C13: 107-120.

Dyer, R.S. and W.E. Howell (1982) Acute triethyltin exposure: effects on the visual evoked potential and hippocampal afterdischarge. Neurobehav. Toxicol. Teratol., 4: 259-266.

Dyer, R.S. and W.E. Howell (1982) Triethyltin: Ambient temperature alters visual system toxicity. Neurobehav. Toxicol. Teratol. 4:267-271.

Dyer, R.S., T.L. Deshields and W.F. Wonderlin (1982) Trimethyltin-induced changes in gross morphology of the hippocampus. Neurobehav. Toxicol. Teratol., 4:141-147.

Dyer, R.S., W.E. Howell and W.F. Wonderlin (1982) Visual system dysfunction following acute trimethyltin administration. Neurobehav. Toxicol. Teratol. 4:191-195.

Dyer, R.S., W.E. Howell and R.C. MacPhail (1981) Dopamine depletion slows retinal transmission. Exp. Neurol., 71: 326-340.

Dyer, R.S. and H.S. Swartzwelder (1978) Sex and strain differences in the visual evoked potentials of albino and hooded rats. Pharmac. Biochem. Behav. 9: 301-306.

Ebe, M., I. Honma and Y. Ishiyama (1972) Laminal analysis of visual evoked potentials and unit discharges on visual cortex of albino rat. Tohoku J. exp. Med. 108:39-54.

Faingold, C.L. (1978) Brainstem reticular formation mechanisms subserving generalized seizures: effects of convulsants and anticonvulsants on sensory evoked responses. Prog. Neuro-Psychopharmacol. 2: 401-422.

Fleming, D.E., D.E. Shearer and D.J. Creel (1974) Effect of pharmacologically induced arousal on the evoked potential in the

unanesthetized rat. Pharmacol. Biochem. Behav. 2: 187-192.

Fox, D.A., J.P. Lewkowski and G.P. Cooper (1977) Acute and chronic effects of neonatal lead exposure on development of the visual evoked response in rats. Toxicol. appl. Pharmacol. 40:449-461.

Forbes, W.B., O. Resnick, W.C. Stern, J.D. Bronzino and P.J. Morgane. (1975) The effect of chronic protein malnutrition on transcallosal evoked responses in the rat. Dev. Psychobiol. 8: 503-509.

Gaarskjaer, F.B. (1978) Organization of the mossy fiber system in the rat studied in extended hippocampi I. terminal area related to number of granule and pyramidal cells. J. Comp. Neurol., 178: 49-72.

Guilbaud, G., D. Menetrey and J.L. Oliveras (1972) Control exerted during sleep by primary cortical areas upon different sensory afferents. Electroencephalog. clin. Neurophysiol. 33: 15-21.

Halgren, E. N. K. Squires, C. L. Wilson, J. W. Rohrbaugh, T. L. Bubb and P. H. Crandall (1980) Endogenous potentials generated in the human hippocampal formation and amygdala by infrequent events. Science 210:803-805.

Hasan, Z., L. Zimmer and D. Woolley (1984) Time course of the effects of trimethyltin on limbic evoked potentials and distribution of tin in blood and brain in the rat. Neurotoxicol. 5: xxx-xxx.

Hatotani, N. and P.S. Timiras, (1967) Influence of thyroid function on the postnatal development of the transcallosal response in the rat. Neuroendocrinology, 2: 147-156.

Hetzler, B.E. and K.E. Oaklay (1981) Dose effects of pentobarbital on evoked potentials in visual cortex and superor colliculus of the albino rat. Neuropharmacol., 20: 969-978.

Howell, W.E. (1978) A neurobehavioral evaluation of the prenatal toxicity of n-hexane in rats. (unpublished doctoral dissertation).

Janssen, R., W.K. Boyes and R.S. Dyer (1983) Effects of chlordimeform on the brainstem auditory evoked response in rats. in A.W. Hayes, R.C. Schnell and T.S. Miya (eds) Developments in the Science and Practice of Toxicology. Elsevier, North Holland, 533-536.

Jordan, T.C. and G.A. Clark (1983) Early undernutrition impairs hippocampal long-term potentiation in adult rats. Behavioral Neuroscience 97: 319-322.

Joseph, R., N.M. Forrest, D. Fiducia, P. Como and J. Siegel (1981) Electrophysiological and behavioral correlates of arousal. Physiol. Psychol. 9: 90-95.

Joy, R. (1985) Kindling as a tool in neurotoxicology. Fund. appl. Toxicol. x: xxx-xxx.

Landfield, P.W., J.L. McGaugh and G. Lynch (1978) Impaired synaptic potentiation processes in the hippocampus of aged, memory-deficient rats. Brain Research 150: 85-101.

Lopes da Silva, F., and A. Van Rotterdam (1982) in E. Niedermeyer and F. Lopes da Silva. Electroencephalography, Urban and Schwarzenberg: Baltimore, pp 15-26.

Lorente de No, R. (1934) Studies on the structure of the cerebral cortex. II. Continuation of the study of the ammonic system. J. Psychol. Neurol. 46: 113-177.

Masukawa, L.M., L.S. Benardo and D.A. Prince (1982) Variations in electrophysiological properties of hippocampal neurons in different subfields. Brain Res. 242: 341-344.

Matthews, W.D., G.P. McCafferty and P.E. Setler (1981) An Electrophysiological model of GABA-mediated neurotransmission. Neuropharmacol. 20: 561-565.

Montero, V.M.(1973) Evoked responses in the rat's visual cortex to contralateral, ipsilateral and restricted photic stimulation. Brain Research, 53: 192-196.

Myers, R.D. (1981) Alcohol's effect on body temperature: hypothermia hyperthermia or poikilothermia. Brain Res. Bull. 7:209-220.

Myslobodsky, M.S. and M. Morag (1981) Suppression by sodium valproate of |-vinyl GABA-induced facilitation of visual evoked potentials in rats. Electroencephalog. clin. Neurophysiol., 52: 445-450.

Myslobodsky, M.S. and M. Morag (1981) Pharmacologic analysis of sodium valproate-induced suppression of secondary components of visual evoked potentials in albino rats. Pharmacol. Biochem. Behav. 15: 681-685.

O'Brien, J. H. (1982) P300 wave elicited by a stimulus - change paradigm in acutely prepared rats. Physiol. Behav. 28:711-713.

Okada, Y. C., L. Kaufman and S. J. Williamson (1983) The hippocampal formation as a source of the slow endogenous potentials. Electroencephalog. Clin. Neurophysiol. 55:417-426.

O'Keefe, J. and L. Nadel (1978) The hippocampus as a cognitive map. Clarendon Press: Oxford.

Otto, D.A., V.A. Benignus, K.E. Muller and C.N. Barton (1981) Effects of age and body lead burden on CNS function in young children. I. Slow cortical potentials. Electroencephal. clin. Neurophysiol. 52: 229-239.

Pickworth, W.B., L.G. Sharpe and W.R. Martin (1977) Transcallosally evoked potentials and the EEG in the decerebrate dog: actions of tryptaminergic dopaminergic and adrenergic agonists. Electroencephalog. clin. Neurophysiol., 42: 809-816.

Pirch, J.H., M.J. Corbus and T.C. Napier (1981) Auditory cue preceding intracranial stimulation induces event-related potential in rat frontal cortex: alterations by amphetamine. Brain res 7:399-404.

Preston, R.J., S.G. Waxman and J.D. Kocsis (1983) Effects of 4-aminopyridine on rapidly and slowly conducting axons of rat corpus callosum. Exper. Neurol. 79: 808-820.

Rabe, L.S., L. Moreno, B.M. Rigor and N. Dafny (1980). Effects of halothane on evoked field potentials recorded from cortical and subcortical nuclei. Neuropharmacol., 19: 813-825.

Ray, D.E. (1981) Electroencephalographic and evoked response correlates of trimethyltin induced neuronal damage in the rat hippocampus. J. appl. Toxicol., 3:145-148.

Rebert, C.S. (1972) Cortical and subcortical slow potentials in the monkeys brain during a preparatory interval. Electroenchephalogy clin. Neurophysiol. 33: 389-402.

Rebert, C.S. (1980) The brain stem auditory evoked response as a tool in neurobehavioral toxicology and medicine. Prog. Brain Res., 54: 458-462.

Rebert, C.S. (1983) Multisensory evoked potentials in experimental and applied neurotoxicology. Neurobeh. Toxicol. Teratol. 5: 659-671.

Rebert, C.S., P.W. Houghton, R.A. Howd, and G.T. Pryor (1982). Effects of hexane on the brainstem auditory response and caudal nerve action potential. Neurobehav. Toxicol. Teratol., 4: 75-89.

Rebert, C.S., S.S. Sorenson, R.A. Howd and G.T. Pryor (1983) Toluene- induced hearing loss in rats evidenced by the brainstem auditory evoked response. Neurobehav. Toxicol. Teratol., 5: 69-76.

Regan, D. (1972) Evoked potentials. Chapman and Hall, London.

Rose, G.H. and D.B. Lindsley (1968). Development of visually evoked potentials in kittens: specific and nonspecific responses. J. Neurophysiol., 31: 607-623.

Ruth, R.E., T.J. Collier and A. Routtenberg (1982) Topography between the entorhinal cortex and the dentate septotemporal axis in rats. I. Medial and intermediate entorhinal projectory cells. J. Comp. Neurol. 209: 69-78.

Rutledge, L.T. (1965) Facilitation: electrical response enhanced by conditional excitation of cerebral cortex. Science, 148: 1246-1248.

Rutledge, L.T. and T.T. Kennedy (1960) Extracallosal delayed responses to cortical stimulation in chloralosed cat. J. Neurophysiol., 23: 188-196.

Schaefer, C.F., C.J. Kreinick and J.S. Schwartzbaum (1974) Behavioral reactivity, appetitive behavior, and visual evoked potentials to photic stimuli following amygdaloid lesions in rats. J. comp. physiol. Psychol., 86: 793-811.

Schanzer, M., E.D. Jacobson and N. Dafny (1978). A neurophysiological approach as a tool to study the effect of drugs on the central nervous system. III. effect of prostaglandin on seven brain sites. Exp. Neurol., 60: 56-67.

Scheinberg, L. C., J. M. Taylor, I. Herzog, and S. Mandell (1966) Optic and peripheral nerve response to triethyltin intoxication in the rabbit: Biochemical and ultrastructural studies. J. Neuropathol. exp. Neurol. 25:202-213.

Schwartzbaum, J.S., L. Ide-Johanson and J. Belgrade (1974) Comparative effects of scopolamine and amphetamine upon behavioral reactivity and visual evoked potentials to flashes in rats. J. comp. physiol. Psychol., 86: 1044-1051.

Schwartzbaum, J.S. and C.J. Kreinick (1973) Interrelationships of hippocampal electroencephalogram, visually evoked response and behavioral reactivity to photic stimuli in rats. J. comp. physiol. Psychol. 85: 479-490.

Schwartzbaum, J.S. and C.J. Kreinick (1974) Visual evoked potentials during appetitive behavior after septal lesions in rats. J. comp. physiol. Psychol. 86: 509-522.

Schwartzbaum, J.S., C.J. Kreinick and M.S. Levine (1972) Behavioral reactivity and visual evoked potentials to photic stimuli following septal lesions in rats. J. comp. physiol. Psychol. 80: 123-142.

Seggie, J. and M. Berry (1972) Ontogeny of interhemispheric evoked potentials in the rat: significance of myelination of the corpus callosum. Exp. Neurol. 35: 215-232.

Shearer, D.E., D.E. Fleming and R.E. Dustman (1978) Effects of dipropylacetic acid on late components of the photically evoked potential and afterdischarge in rat. Pharmacol. Biochem. Behav. 8: 501-504.

Standage, G.P., D.E. Fleming and E.D. Bigler (1981) Thalamocortical coupling and component properties of visually evoked afterdischarge. Brain Res. Bull., 7: 89-92.

Swadlow, H.A. (1982) Impulse conduction in the mammalian brain: physiological properties of individual axons monitored for several months. Science, 218: 911-913.

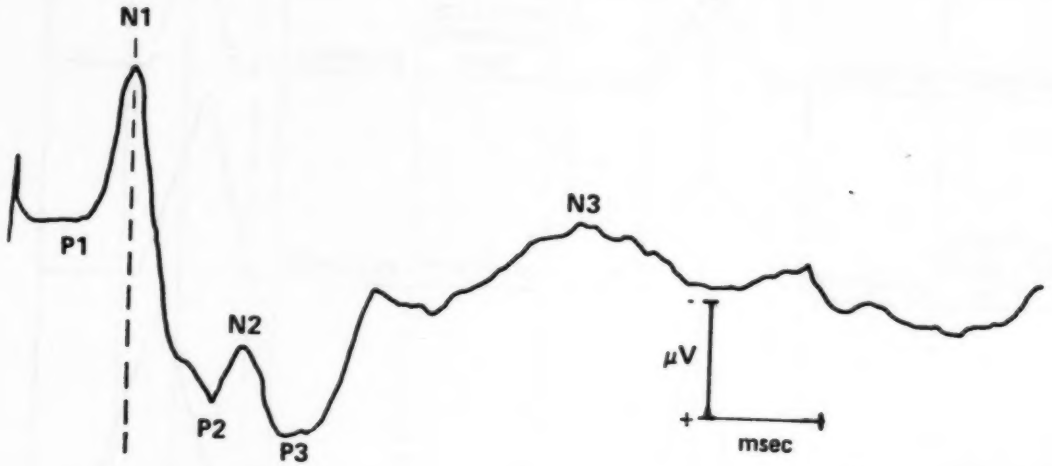
Swanson, L.W. (1979) The hippocampus - new anatomical insights. Trends Neurosci. 2: 9-12.

Torres, F. and J.S. Warner (1962). Some characteristics of delayed responses to photic stimuli in the cat. Electroencephalog. clin. Neurophysiol., 14: 654-666.

Verzeano, M. (1973) The study of neuronal networks in the mammalian brain. In R.F. Thompson, and M.M. Patterson, (Eds.): Bioelectric recording techniques. Part C. Cellular processes and brain potentials. Academic Press, N.Y.

Westerfield, M., R.W. Joyner and J.W. Moore (1978) Temperature-sensitive conduction failure at axon branch points. J. Neurophysiol., 41: 1-8.

Winneke, G. (1979) Modification of visual evoked potentials in rats after long term blood lead elevation. Activ. nerv. sup. 21: 282-284.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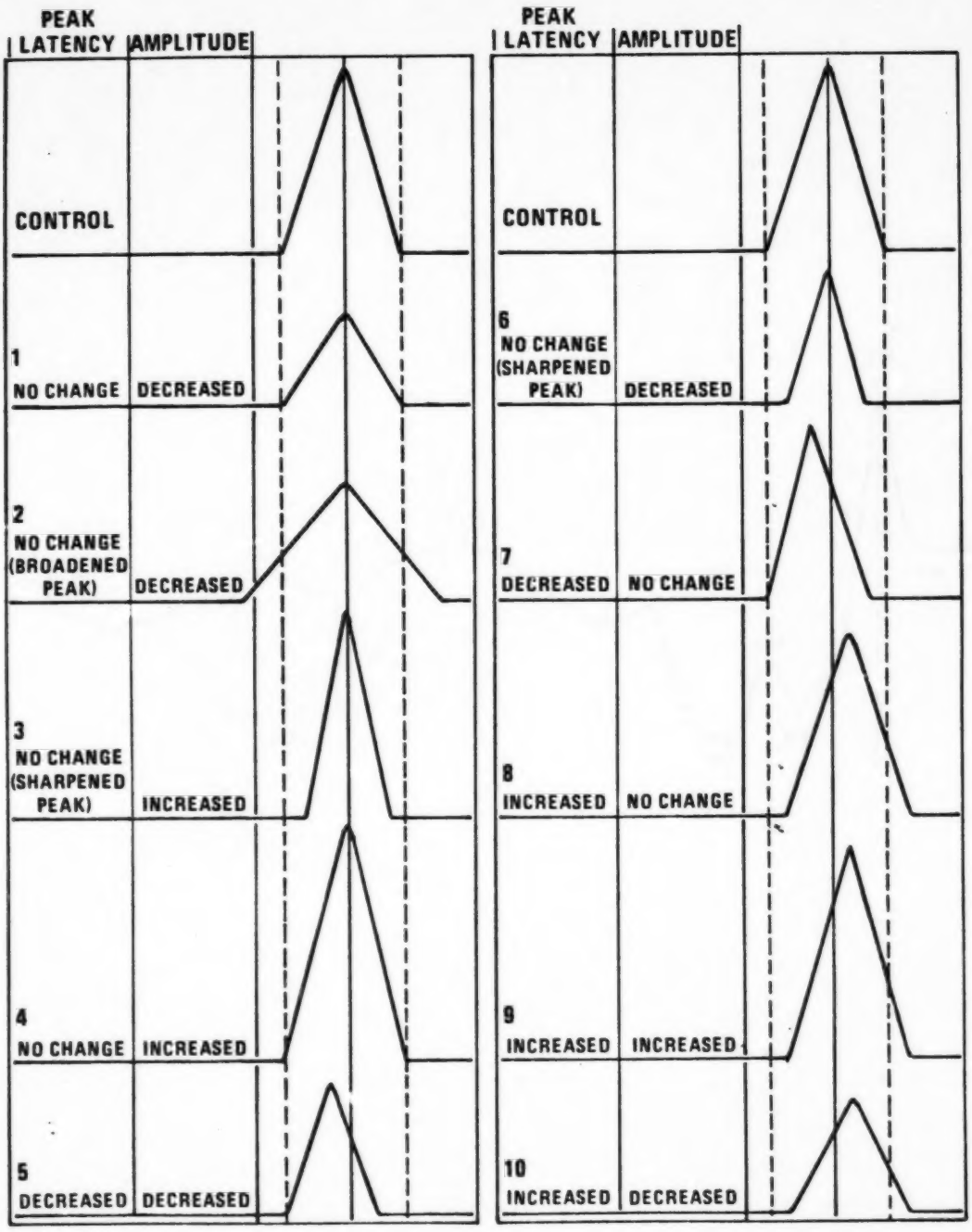
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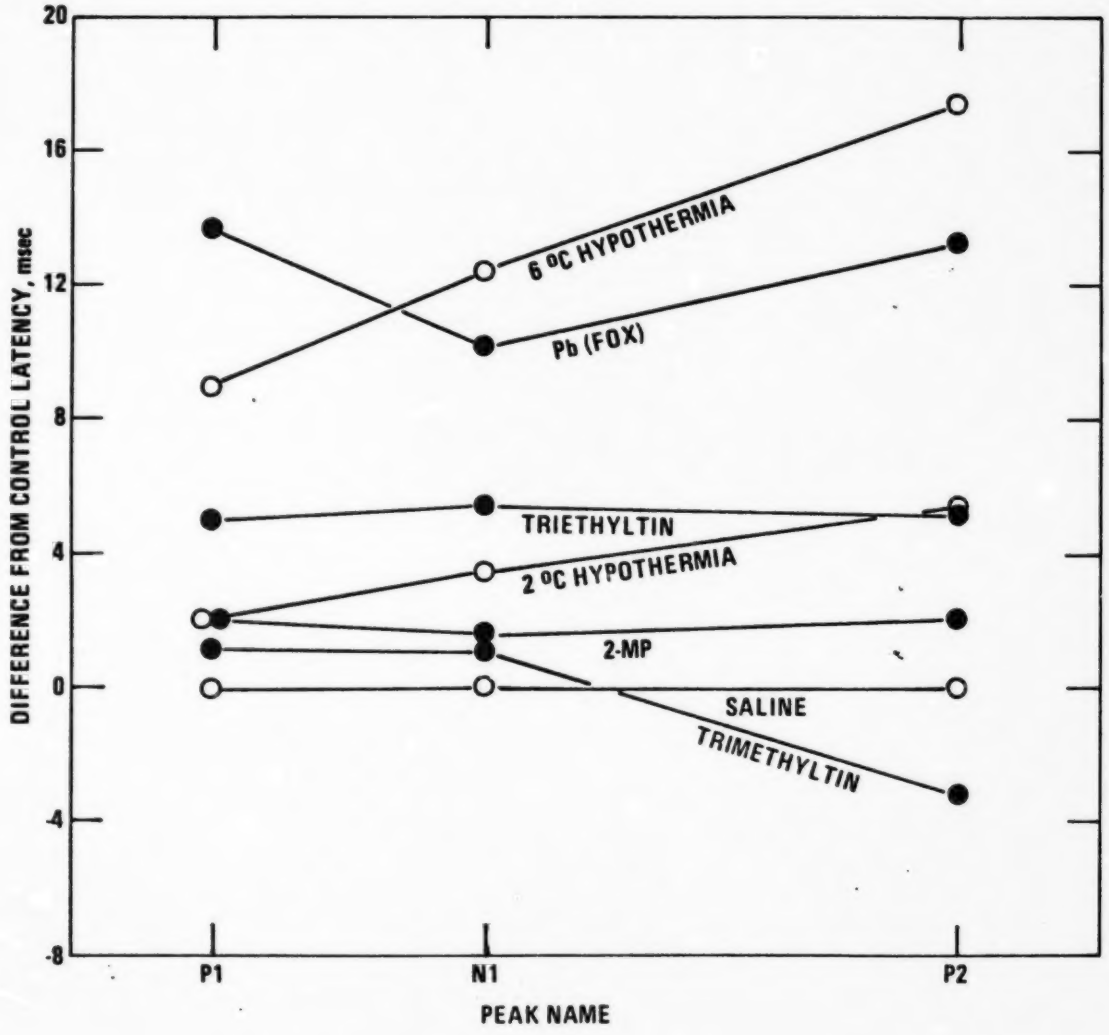
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